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Effect of α-blockade on liver regeneration after carbon tetrachloride intoxication in the rat

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Carbon tetrachloride (CCl_4) has long been used as a typical hepatotoxin to cause hepatocellular necrosis [1]. However, only limited studies have been made on the successive process after injury as well as on the defensive mechanism of the experimental animal. Recently, we reported [2] that liver regeneration takes place after administration of CCl_4 by showing increased activities of thymidylate synthetase (TS; EC 2.1.1.45) and thymidine kinase (TK; EC 2.7.1.21), key enzymes of DNA synthesis. The activities of these enzymes determine the amount of DNA synthesis in liver regeneration following partial hepatectomy [3]. Many drugs and ablation of endocrine organs interfere with liver regeneration by regulating the induction of TS and TK [4–10]. Based on these *in vivo* studies, we have reached the conclusion that hepatic DNA synthesis after partial hepatectomy is regulated by catecholamines through liver α_1-receptors. On the other hand, no systematic investigations have been done on the controlling mechanism of DNA synthesis after CCl_4 intoxication, although administration of the drug is often employed as an alternative method of partial hepatectomy to cause hepatocellular proliferation.

The present experiments were designed to elucidate the effect of the α-blocker on hepatic DNA synthesis after extensive damage by CCl_4 on the basis of the activities of TS and TK, similar to our previous studies on liver regeneration caused by partial hepatectomy.

Materials and Methods

Male Wistar rats (190–250 g) were used in this study. CCl_4 in corn oil (1:1, v/v) was administered through an intragastric tube in a single dose of 0.4 mL/100 g body weight. The control group received an equal volume of oil without CCl_4. Phenoxybenzamine (10 mg/kg, i.p.) in 50% propylene glycol was injected 24 hr after the administration of CCl_4. Propranolol (20 mg/kg, i.p.) in 50% propylene glycol also was given 24 hr after CCl_4 intoxication. All animals had free access to food and water.

The rats were killed under ether anesthesia 48 hr after CCl_4 ingestion. Blood was collected by puncturing the abdominal aorta. The liver was perfused *in situ* with saline. The excised liver was homogenized in 50 mM Tris–HCl buffer, pH 7.5, containing 0.25 M sucrose and 10 mM β-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride and 1 mM EDTA (the tissue:buffer ratio was 1:4). A liver homogenate was centrifuged at 36,000 *g* for 30 min at 4°, and the supernatant fraction was used as the enzyme preparation. TS and TK activities were determined as described previously [2] by using [5-^{3}H]deoxyuridine monophosphate and [6-^{3}H]thymidine respectively. Protein was measured by the method of Lowry *et al.* [11], using bovine serum albumin as the standard.

The activity of plasma glutamate-oxaloacetate transaminase (GOT; EC 2.6.1.1) was measured spectroscopically utilizing the diagnostic kits from Boehringer Mannheim and is expressed as I.U./L [12–14].

Statistical analyses of data were done with Student's *t*-test.

Results and Discussion

At 48 hr after the administration of CCl_4, the plasma GOT level was 4878 I.U./L, which was 97 times that of the control (Table 1). The activities of TS and TK 48 hr after CCl_4 administration were 51.2 and 92.8 pmol/mg protein/min, which were 9.7 and 5.3 times the levels of the respective controls (Table 1). These results are consistent with our previous study [2]. In the rats injected with phenoxybenzamine (10 mg/kg, i.p.) 24 hr after CCl_4 intoxication, the plasma level of GOT 48 hr after CCl_4 was similar to the CCl_4 group which received only CCl_4 and oil. This result demonstrates that the α-adrenoceptor antagonist did not influence the degree of liver damage caused by CCl_4. However, the liver TS activity of the rats given phenoxybenzamine was suppressed to about 43% of the CCl_4 group 48 hr after CCl_4 ingestion. The TK level was also depressed by the injection of the α-blocker (Table 1). On the other hand, the injection of propranolol (20 mg/kg, i.p.) 24 hr after CCl_4 intoxication did not prevent the increase of TS and TK activities at 48 hr after CCl_4 administration. The β-adrenoceptor antagonist did not affect the rise of plasma GOT level caused by CCl_4 (Table 1).

The results of this study suggest that α-adrenergic stimu-

Table 1. Plasma GOT and liver thymidylate synthetic enzyme levels 48 hr after CCl_4 intoxication

Treatment	No. of rats	Plasma GOT (I.U./L)	Liver TS (pmol/min/mg protein)	Liver TK (pmol/min/mg protein)
Control	7	49 ± 10	5.3 ± 2.1	17.5 ± 3.8
CCl_4	6	4878 ± 1137*	51.2 ± 8.4*	92.8 ± 14.3*
CCl_4 + phenoxybenzamine	6	4396 ± 975*	22.0 ± 5.3*†	45.3 ± 13.0*†
CCl_4 + propranolol	5	4630 ± 1054*	54.7 ± 11.8*	106.6 ± 21.3*

The control group received an equal volume of oil without CCl_4. Phenoxybenzamine (10 mg/kg, i.p.) or propranolol (20 mg/kg, i.p.) was injected 24 hr after CCl_4 (4 mL/kg) administration. All animals were killed 48 hr after CCl_4 with corn oil or corn oil only (control), and the assay of the enzymatic activities was carried out as described in Materials and Methods. Values are means ± SE.

* Significantly different ($P < 0.05$) from the control (*t*-test).

† Significantly different ($P < 0.05$) from the CCl_4 group.

lation regulates DNA synthesis in regenerating rat liver following CCl_4 administration. Little is known about whether the regulating mechanism of liver regeneration is similar following various types of liver damage. Our results suggest that the controlling role of catecholamines via the α-receptor is common in regenerating liver after CCl_4 intoxication and partial hepatectomy.

CCl_4 is a typical hepatotoxin causing centrilobular necrosis [1]. As a result of extensive studies, the initial event in the rat given CCl_4 has been established to be lipid peroxidation of the endoplasmic reticulum of the liver cell mediated by cytochrome P450 [15]. CCl_4 induces a marked necrosis during the initial 24 hr, when TS and TK levels do not increase [2]. An active hepatic proliferation involving the rise of TS and TK activities reaches its maximum at 48–72 hr after CCl_4 administration [2]. In this study, phenoxybenzamine injected at 24 hr suppressed the rise of TS and TK activities at 48 hr after CCl_4 ingestion. Therefore, the stimulation of hepatic α-receptors seems to occur around or after 24 hr following CCl_4 intoxication.

In summary, liver regeneration after administration of carbon tetrachloride was monitored by measuring thymidylate synthetase (TS) and thymidine kinase (TK) activities. The administration of an α-blocking agent, phenoxybenzamine, 24 hr after CCl_4 intoxication suppressed the rise of TS and TK, which occurred 48 hr after CCl_4 ingestion. This result suggests that liver regeneration after CCl_4 intoxication or partial hepatectomy is regulated in a similar way by catecholamines via an α-receptor.

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Diffusion and absorption of (−)sulpiride and raclopride after intracerebral administration in the rat

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Intracerebral administration of drugs has often been used in animal studies to study localization of functions within the CNS, including two recent reports from this laboratory [1, 2]. The implicit assumption being that the injection is confined within the intended target in contrast to general effects produced by systemic administration. In the present report, we have investigated the diffusion within the rat striatum of two dopamine (DA) D_2 selective antagonists, (−)sulpiride [3] and raclopride [4], by estimating DA turnover at the site of injection and in adjacent brain areas.